PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
P38578WO/NCB		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2005/001108	24.03.2005	27.03.2004

International Patent Classification (IPC) or national classification and IPC
INV. C07K16/44 A61K39/40

Applicant
HAPTOGEN LTD et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 9 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08.02.2006	10.08.2006

Name and mailing address of the international preliminary examining authority:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized officer

Covone-van Hees, M.G

Telephone No. +31 70 340-4416

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2005/001108

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-39 as originally filed

   Claims, Numbers

   1-40 filed with telefax on 08.02.2006

   Drawings, Sheets

   1/8-8/8 as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-39 as to I.A.

because:

☒ the said international application, or the said claims Nos. 1-39 as to industrial applicability relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*.

☐ no international search report has been established for the said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-40
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-40
Industrial applicability (IA)	Yes:	Claims	40
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 1-13 and 27-39 (as far as an in-vivo method is concerned) and 14-26 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO2004014423
D2: McGrath et al. (2004)
D3: D'Argenio et al. (2002)

2. **Article 6 PCT**

   1. The subject-matter of independent claim 14 refers to a method to treat a disease defined i.a. by a mechanism of action, namely "as to cause an imbalance in the ratio of homoserine lactone (HL) signal molecule to quinolone signal (QS) signal molecule in the environment of the gram-negative bacteria" rather than clearly defining the disease to be treated. The intended limitations are therefore not clear from this claim, contrary to the requirements of Article 6 PCT. Accordingly this definition cannot be considered as a limiting technical feature on which novelty (33(2) PCT) and inventive step (33(3) PCT) may be established.

3. **Novelty** (Article 33(2) PCT

   1. Claim 1: D1 studies methods to treat an infection of gram-negative bacteria in patients by regulating the concentration of bacterial cell signalling molecules by means of an antibody binding to a lactone or lactone-derived signal molecule (see pg.8 l.11-18). The ability of anti-lactone (derived) antibodies to protect

animals from Pseudomonas aeruginosa infection (=gram-negative bacteria) has been shown on an in vitro slow-kill assay using a nematode (see pg.33 Ex.2). It appears from Figure 8 that the antibodies prevent P. aeruginosa from killing the nematodes through the sloww-killing mechanism which takes place wihin 3-4 days. Therefore D1 proves that anti-lactone (derived) antibodies are effective in rendering P. aeruginosa cells unable to infect the worm. Even if D1 is not explicitly mentioning "autolysis" or "causing imbalance in the ration HL/QS, these are a implicit properties of the anti-lactone antibodies known in the art, which are the same used in the present invention. The mechanism of action of the lactone is implicit to the use of it render bacteria non-infective. These features cannot therefore reestablish novelty of a known method, where the only distinguishing features are the mechanism of action of a known product in a known method. For this reason D1 is detrimental to the novelty (Article 33(2) PCT) of the subject-matter of independent claim 1.

2. Dependent claims 2-10 and independent claim 14 (see also point 2.1 above) are also anticipated by the disclosure of D1 (Article 33(2) PCT).
3. The lactone (derived) molecules are i.e. homoserine molecule having general formula showed at pg.13 l.1-18. D1 anticipates the subject-matter of claims 15-23 and independent claim 40.
4. The anti-lactone (derivative) antibody may be monoclonal, polyclonal or derivatives thereof which are capable of binding to antigen (see pg.8 l.20-pg.9 l.12). D1 anticipates the subject-matter of dependent claim 11, independent claim 27 and dependent claims 28-37.
5. In the preferred embodiments antibodies are generated by screening a phage display library to produce scAbs and deposited under the accession numbers: NCIMB-41167, NCIMB-41168, NCIMB-41169, NCIMB-41170 (see pg.17 l.12-17). D1 anticipates the subject-matter of claims 12,13,25,26,38,39.
6. Summing up: The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-40 is not new in the sense of Article 33(2) PCT.

4. **Article 6 and 33(3) PCT**

1. Claim 1 broadly refers to a method of causing autolysis .... using generic anti-lactone (derived) Ab .... causing an imbalance in the ratio HL/QS. However the

disclosures of D2 and D3 cast doubt if present claim is supported and solves the problem over its whole broad scope.

2. D2 explains that QS production depends on the ratio of the two quorum sensing signals N-(3-oxododecanoyl)-$_L$-homoserine (3-oxo-C_{12}-HSL) and N-(butanoyl)-$_L$-homoserine lactone (C_4-HSL), whereby (3-oxo-C_{12}-HSL) induces the production of QS (see the whole article, especially pg.33 left-hand column l.28-46).
3. Moreover, D3 studies P. aeruginosa mutants with visible lysis, correlating with the level of the Pseudomonas quinolone signal (QS), "an extracellular signal that interacts with the quorum sensing regulatory hierarchy" (see pg. 6481 right-hand column l.23- left-hand column l.7). In particular the authors of present article correlate autolysis of the mutant strains with overproduction of QS (see pg.6485 right-hand column l.19-30; pg.6487 left-hand column last three lines and fig.9).
4. According to the teaching of these articles therefore, it appears that to target any lactone (derivative) molecule may not cause the desired effect, namely the lysis of gram-negative bacteria, since autolysis is linked to hight level of QS and the different lactone (derived) molecules have different effect on QS level.
5. Consequently, it seems that only the anti-lactone (derived) Ab exemplified in the application may have the desired effect, and therefore the subject-matter of claim 1 lacks support (Article 6 PCT)and does not to solve the problem of the application (Article 33(3) PCT) over its whole scope.

5. For the assessment of the present claims 1-13 and 27-39 (as far as an in-vivo method is concerned) and 14-26 and 40 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

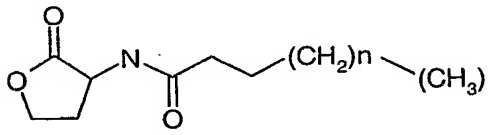
Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D3 is not mentioned in the description, nor are these documents identified therein.

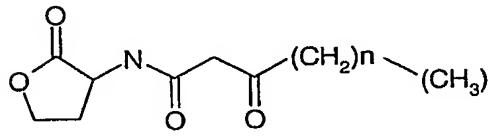
CLAIMS

1. A method of causing autolysis of a population of gram-negative bacteria, said method comprising administration to the population of an antibody to a lactone or lactone-derived signal molecule secreted by gram-negative bacteria so as to cause an imbalance in the ratio of homoserine lactone (HL) signal molecule to quinolone signal (QS) signal molecule in the environment of the population of the gram-negative bacteria.

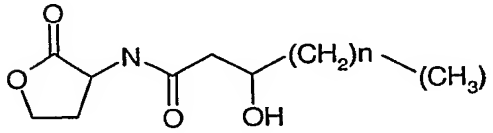
2. A method as claimed in claim 1, in which the homoserine lactone (HL) signal molecule is a homoserine lactone molecule with a formula selected from the group consisting of:



Formula (I)



Formula (II)



Formula (III)

where n = 0 to 12.

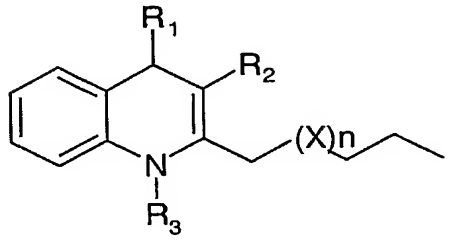
3. A method as claimed in claim 2, in which the homoserine lactone molecule of general formula (I) is *N*-butanoyl-L-homoserine lactone (BHL) where n = 0, *N*-dodecanoyl-L-homoserine lactone (dDHL) where n = 8 and *n*-tetradecanoyl-L-homoserine lactone (tDHL) where n = 10.

4. A method as claimed in claim 2, in which the homoserine lactone molecule of general formula (II) is *N*-(-3-oxododecanoyl)-L-homoserine lactone (OdDHL) where n = 8 or *N*-(-3-oxohexanoyl)-L-homoserine lactone (OHHL) where n = 2.

5. A method as claimed in claim 2, in which the homoserine lactone molecule of general formula (III) is *N*-(-3-hydroxybutanoyl)-L-homoserine lactone (HBHL) where n = 0.

6. A method as claimed in claim 2, in which the lactone signal molecule is OdDHL and/or BHL.

7. A method as claimed in any preceding claim, in which the quinolone signal (QS) signal molecule is a molecule of general formula (IV):



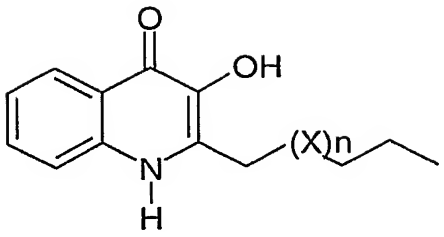
where n is 1 to 7,

R_1 is =O, or –H,

R_2 is –OH, or –H, and

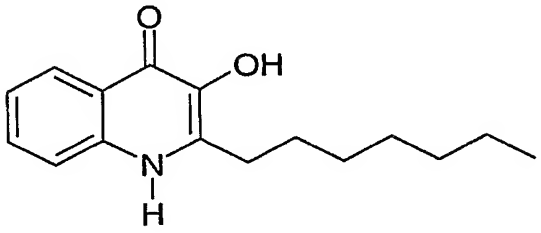
R_3 is –H, or alternatively, the nitrogen atom (N) is unsubsituted.

8. A method as claimed in claim 7, in which the quinolone signal molecule of general formula (IV) is



2-acyl-3-hydroxy-4-quinolone

9. A method as claimed in claim 8, in which the 2-acyl-3-hydroxy-4-quinolone is 2-heptyl-3-hydroxy-4-quinolone



10. A method as claimed in any preceding claim, in which the gram negative bacteria is *Pseudomonas aeruginosa* and the ratio of bacterial signal molecules is acyl-homoserine lactone (AHL) signal molecule of formula (I) to *Pseudomonas* quinolone signal (PQS) signal molecule.

11. A method as claimed in any preceding claim, in which the antibodies are monoclonal or polyclonal antibodies, or fragments thereof.

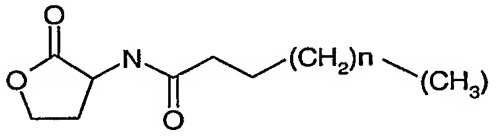
12. A method as claimed in claim 11 in which the antibody fragments are single chain antibody fragments (scAbs).

13. A method as claimed in claim 12, in which the single-chain antibodies (scAbs) are G3H5, G3B12, G3G2 and/or G3H3 deposited as NCIMB-41167, NCIMB-41168, NCIMB-41169, NCIMB-41170, respectively.

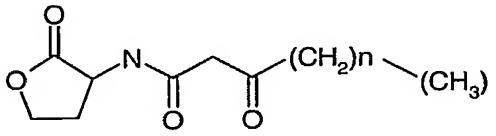
14. A method for the treatment of an infection of gram-negative bacteria in a subject, said method comprising administration to the subject of an antibody to a

lactone or lactone-derived signal molecule secreted by gram-negative bacteria so as to cause an imbalance in the ratio of homoserine lactone (HL) signal molecule to quinolone signal (QS) signal molecule in the environment of the gram-negative bacteria.

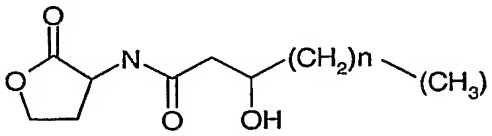
15. A method as claimed in claim 14, in which the homoserine lactone (HL) signal molecule is a homoserine lactone molecule with a formula selected from the group consisting of:



Formula (I)



Formula (II)



Formula (III)

where n = 0 to 12.

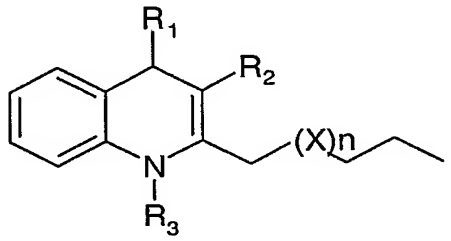
16. A method as claimed in claim 15, in which the homoserine lactone molecule of general formula (I) is *N*-butanoyl-L-homoserine lactone (BHL) where n = 0, *N*-dodecanoyl-L-homoserine lactone (dDHL) where n = 8 and *n*-tetradecanoyl-L-homoserine lactone (tDHL) where n = 10.

17. A method as claimed in claim 15, in which the homoserine lactone molecule of general formula (II) is *N*-(-3-oxododecanoyl)-L-homoserine lactone (OdDHL) where n = 8 or *N*-(-3-oxohexanoyl)-L-homoserine lactone (OHHL) where n = 2.

18. A method as claimed in claim 15, in which the homoserine lactone molecule of general formula (III) is *N*-(-3-hydroxybutanoyl)-L-homoserine lactone (HBHL) where n = 0.

19. A method as claimed in claim 15, in which the lactone signal molecule is OdDHL and/or BHL.

20. A method as claimed in any one of claims 14 to 19, in which the quinolone signal (QS) signal molecule is a molecule of general formula (IV):



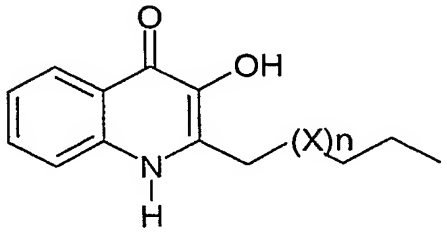
where n is 1 to 7,

R_1 is =O, or –H,

R_2 is –OH, or –H, and

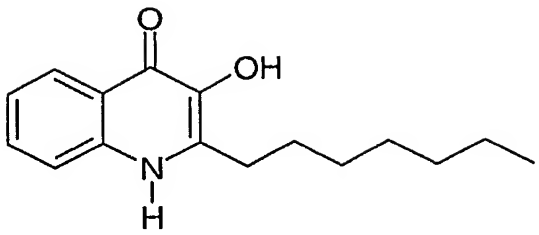
R_3 is –H, or alternatively, the nitrogen atom (N) is unsubsituted.

21. A method as claimed in claim 20, in which the quinolone signal molecule of general formula (IV) is



2-acyl-3-hydroxy-4-quinolone

22. A method as claimed in claim 21, in which the 2-acyl-3-hydroxy-4-quinolone is 2-heptyl-3-hydroxy-4-quinolone



23. A method as claimed in any one of claims 14 to 22, in which the gram negative bacteria is *Pseudomonas aeruginosa* and the ratio of bacterial signal molecules is acyl-homoserine lactone (AHL) signal molecule of formula (I) to *Pseudomonas* quinolone signal (PQS) signal molecule.

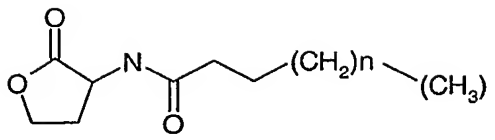
24. A method as claimed in any one of claims 14 to 23, in which the antibodies are monoclonal or polyclonal antibodies, or fragments thereof.

25. A method as claimed in claim 24 in which the antibody fragments are single chain antibody fragments (scAbs).

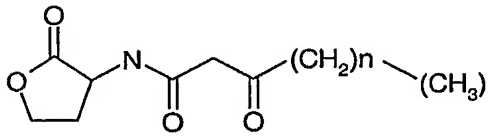
26. A method as claimed in claim 25, in which the single-chain antibodies (scAbs) are G3H5, G3B12, G3G2 and/or G3H3 deposited as NCIMB-41167, NCIMB-41168, NCIMB-41169, NCIMB-41170, respectively.

27. The use of an antibody to a lactone or lactone-derived signal molecule secreted by gram-negative bacteria in causing autolysis of gram-negative bacteria.

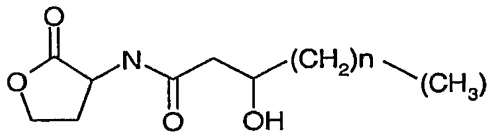
28. A use as claimed in claim 27, in which the lactone or lactone-derived signal molecule is a homoserine lactone molecule with a formula selected from the group consisting of:



Formula (I)



Formula (II)



Formula (III)

where n = 0 to 12.

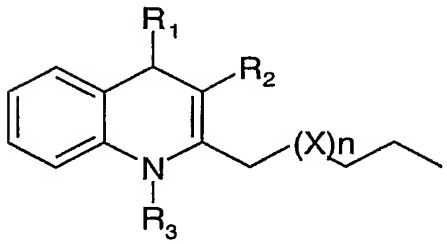
29. A use as claimed in claim 28, in which the homoserine lactone molecule of general formula (I) is *N*-butanoyl-L-homoserine lactone (BHL) where n = 0, *N*-dodecanoyl-L-homoserine lactone (dDHL) where n = 8 and *n*-tetradecanoyl-L-homoserine lactone (tDHL) where n = 10.

30. A use as claimed in claim 28, in which the homoserine lactone molecule of general formula (II) is *N*-(-3-oxododecanoyl)-L-homoserine lactone (OdDHL) where n = 8 or *N*-(-3-oxohexanoyl)-L-homoserine lactone (OHHL) where n = 2.

31. A use as claimed in claim 28, in which the homoserine lactone molecule of general formula (III) is *N*-(-3-hydroxybutanoyl)-L-homoserine lactone (HBHL) where n = 0.

32. A use as claimed in claim 28, in which the lactone signal molecule is OdDHL and/or BHL.

33. A use as claimed in any one of claims 27 to 32, in which the quinolone signal (QS) signal molecule is a molecule of general formula (IV):



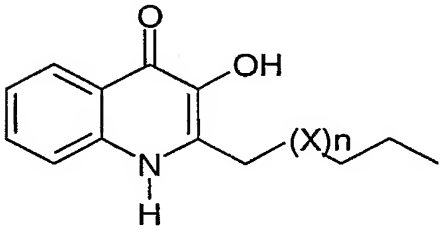
where n is 1 to 7,

R_1 is =O, or –H,

R_2 is –OH, or –H, and

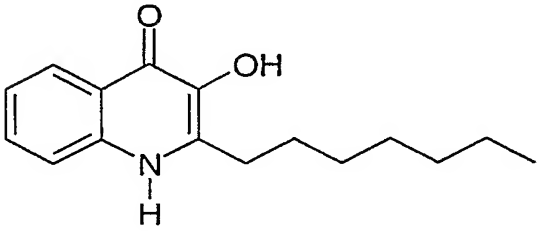
R_3 is –H, or alternatively, the nitrogen atom (N) is unsubsituted.

34. A use as claimed in claim 33, in which the quinolone signal molecule of general formula (IV) is



2-acyl-3-hydroxy-4-quinolone

35. A use as claimed in claim 34, in which the 2-acyl-3-hydroxy-4-quinolone is 2-heptyl-3-hydroxy-4-quinolone



36. A use as claimed in any one of claims 27 to 35, in which the gram negative bacteria is *Pseudomonas aeruginosa* and the ratio of bacterial signal molecules is acyl-homoserine lactone (AHL) signal molecule of formula (I) to *Pseudomonas* quinolone signal (PQS) signal molecule.

37. A use as claimed in any one of claims 27 to 36, in which the antibodies are monoclonal or polyclonal antibodies, or fragments thereof.

38. A use as claimed in claim 37 in which the antibody fragments are single chain antibody fragments (scAbs).

39. A use as claimed in claim 38, in which the single-chain antibodies (scAbs) are G3H5, G3B12, G3G2 and/or G3H3 deposited as NCIMB-41167, NCIMB-41168, NCIMB-41169, NCIMB-41170, respectively.

40. The use of an antibody to a lactone or lactone-derived signal molecule secreted by gram-negative bacteria in the preparation of a medicament for the treatment of an infection of gram-negative bacteria in a subject, in which the antibody causes autolysis of the gram-negative bacteria which infect said subject.